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PRD2009-13

Proposed Registration Decision

RootShield Biological Fungicide, *Trichoderma harzianum* Rifai strain KRL-AG2

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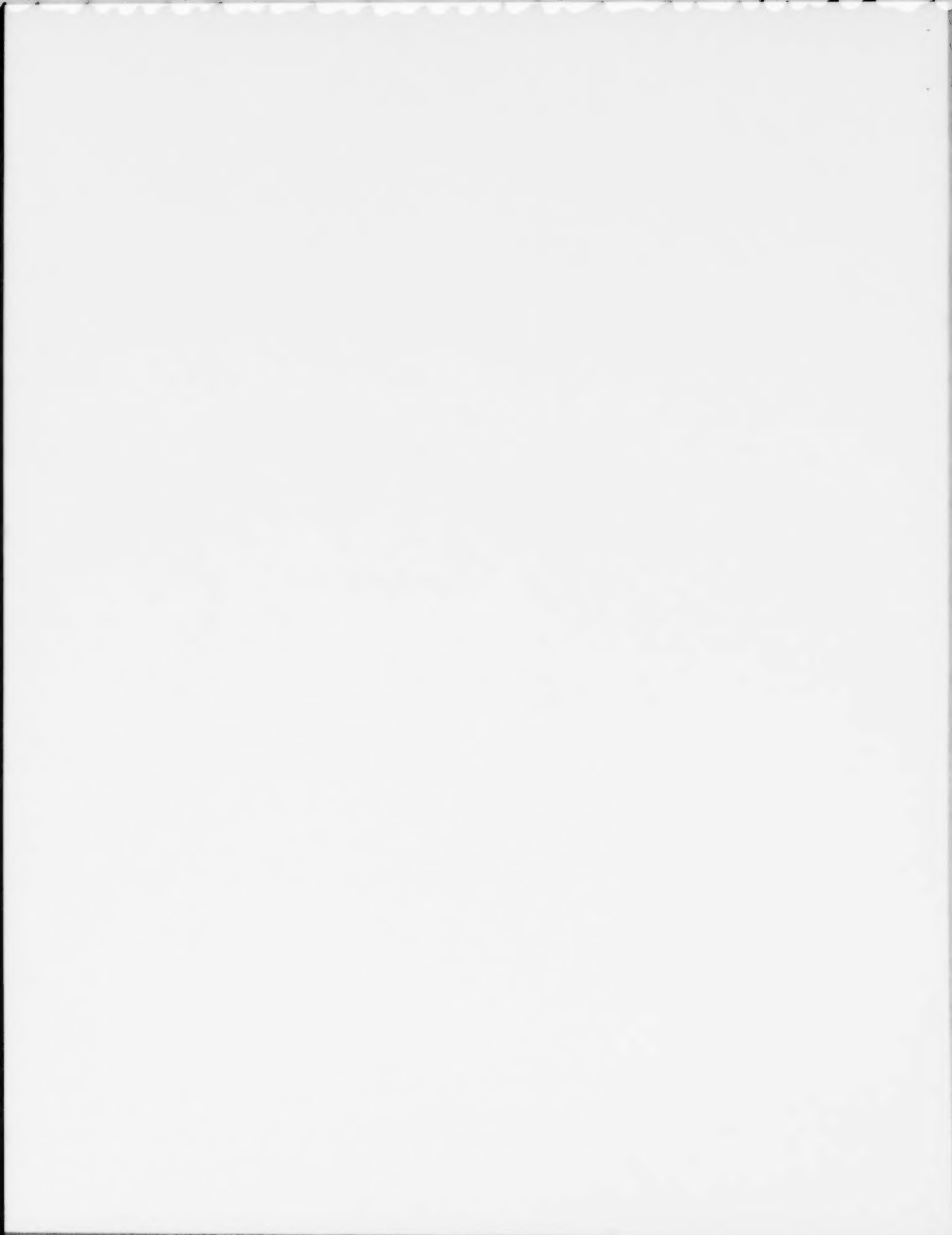
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Overview

Proposed Registration Decision for *Trichoderma harzianum* Rifai strain KRL-AG2

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is proposing amended full registration for the sale and use of RootShield Technical Biological Fungicide, RootShield HC-Biological Fungicide Wettable Powder and RootShield Granules Biological Fungicide containing the technical grade active ingredient *Trichoderma harzianum* Rifai strain KRL-AG2, to control a variety of fungal diseases on greenhouse peppers, tomato and strawberry (in both greenhouses and the field), field lettuce and outdoor nursery plants as well as bean, pea, lima bean, lentil and soybean seeds.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

This Overview describes the key points of the evaluation, while the Science Evaluation provides detailed technical information on the human health, environmental and value assessments of RootShield Technical Biological Fungicide, RootShield HC- Biological Fungicide Wettable Powder and RootShield Granules Biological Fungicide.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable¹ if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its proposed conditions of registration. The Act also requires that products have value² when used according to label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (children, for example) and organisms in the environment (those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects

¹ "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act*.

² "Value" as defined by subsection 2(1) of the *Pest Control Products Act*: "the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (a) efficacy; (b) effect on host organisms in connection with which it is intended to be used; and (c) health, safety and environmental benefits and social and economic impact."

observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the Pesticide and Pest Management portion of Health Canada's website at healthcanada.gc.ca/pmra.

Before making a final registration decision on *Trichoderma harzianum* Rifai strain KRL-AG2, the PMRA will consider all comments received from the public in response to this consultation document.³ The PMRA will then publish a Registration Decision⁴ on *Trichoderma harzianum* Rifai strain KRL-AG2, which will include the decision, the reasons for it, a summary of comments received on the proposed final registration decision and the PMRA's response to these comments.

For more details on the information presented in this Overview, please refer to the Science Evaluation of this consultation document.

What Is *Trichoderma harzianum* Rifai strain KRL-AG2?

Trichoderma harzianum Rifai strain KRL-AG2 is a fungus that protects plants from disease-causing fungal pathogens by secreting cell degrading enzymes and antibiotics and by invading and growing within pathogenic fungi to suppress their growth.

The end-use products RootShield HC-Biological Fungicide Wettable Powder (previously RootShield Drench) and RootShield Granules Biological Fungicide are commercial fungicide products that contain *Trichoderma harzianum* Rifai strain KRL-AG2 as the active ingredient. These products control fungal root disease in soils and foliar disease in ornamental and food crops.

Health Considerations

Can Approved Uses of *Trichoderma harzianum* Rifai strain KRL-AG2 Affect Human Health?

***Trichoderma harzianum* Rifai strain KRL-AG2 is unlikely to affect your health when RootShield Biological Fungicide is used according to the label directions.**

People could be exposed to *Trichoderma harzianum* Rifai strain KRL-AG2 when handling and applying the products. The PMRA considers several key factors when assessing health risks: the microorganism's biological properties (production of toxic byproducts, for example); reports of any adverse incidents; potential to cause disease or toxicity as determined in toxicological studies; and the level to which people may be

³ "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act*.

⁴ "Decision statement" as required by subsection 28(5) of the *Pest Control Products Act*.

exposed relative to exposures already encountered in nature to other isolates of this microorganism.

Toxicological studies in laboratory animals describe potential health effects from large doses for the purpose of identifying any potential pathogenicity, infectivity and toxicity concerns. When *Trichoderma harzianum* strain KRL-AG2 was tested on laboratory animals, there were no signs it caused any significant toxicity or disease.

Residues in Water and Food

Dietary risks from food and water are not of concern.

The *Food and Drugs Act* prohibits the sale of food containing a pesticide residue that exceeds the established maximum residue limit (MRL). Pesticide MRLs are established for *Food and Drugs Act* purposes through the evaluation of scientific data under the *Pest Control Products Act*. Each MRL value determines the maximum concentration in parts per million (ppm) of a pesticide allowed in or on certain foods. Food containing a pesticide residue that does not exceed the established MRL does not pose an unacceptable health risk.

Strains of *Trichoderma harzianum* are common in nature and the use of RootShield Biological Fungicide products to control fungal diseases in crops is not expected to significantly increase natural environmental background levels of this microorganism. Furthermore, when *Trichoderma harzianum* strain KRL-AG2 was administered orally to rats, no signs that it caused toxicity or disease were observed. Secondary metabolites of toxicological significance (i.e., peptaibols) have been shown to be produced by certain naturally occurring strains of *Trichoderma harzianum* (including strain KRL-AG2). However the use of RootShield Biological Fungicide products is not expected to result in a sustained increase in levels of these peptaibols beyond the naturally occurring background levels of those produced by native *Trichoderma harzianum* strains. These metabolites are expected to be short lived in the environment once produced, as they are susceptible to ultraviolet light, high temperatures and various microbial processes in the environment.

The establishment of a MRL is not therefore required for *Trichoderma harzianum* strain KRL-AG2. As well, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Consequently, dietary exposure and risks are minimal to non-existent.

Occupational Risks from Handling RootShield HC-Biological Fungicide Wettable Powder or RootShield Granules Biological Fungicide

Occupational risks are not of concern when RootShield HC-Biological Fungicide Wettable Powder or RootShield Granules Biological Fungicide is used according to label directions, which include protective measures.

Workers using RootShield Fungicide products can come into direct contact with *Trichoderma harzianum* strain KRL-AG2 on the skin, in the eyes or through inhalation. For this reason, the label will specify that users exposed to RootShield Biological Fungicide products must wear gloves, long-sleeved shirts, long pants, a NIOSH approved respirator (with any N, P, R or HE filter), and shoes plus socks.

For bystanders, exposure is expected to be much less than that of handlers and mixer/loaders and is considered negligible. Therefore, health risks to bystanders are not of concern.

Environmental Considerations

What Happens When *Trichoderma harzianum* Rifai strain KRL-AG2 Is Introduced Into the Environment?

Environmental risks are not of concern.

Information on the environmental fate of *Trichoderma harzianum* strain KRL-AG2 suggests that, as a soil microorganism, it is likely to survive in outdoor soil under suitable environmental conditions (in other words, the type of soil, moisture, acidity levels and temperature). Over time, however, the populations of *Trichoderma harzianum* strain KRL-AG2 should return to naturally occurring levels.

There are no published reports of disease associated with *Trichoderma harzianum* strain KRL-AG2 in birds, wild mammals, fish, insects, earthworms, or plants. Furthermore, studies designed to examine the effects of *Trichoderma harzianum* on birds, wild mammals, terrestrial insects, earthworms and soil microorganisms reported no adverse effects.

Value Considerations

What Is the Value of RootShield HC-Biological Fungicide Wettable Powder or RootShield Granules Biological Fungicide?

RootShield HC-Biological Fungicide Wettable Powder and RootShield Granules Biological Fungicide contain a fungus that suppresses:

- root diseases caused by *Pythium* spp., *Rhizoctonia* spp. and *Fusarium* spp. on greenhouse tomatoes, peppers and cucumbers as well as outdoor nursery crops;
- grey mould in strawberries;
- botrytis stem canker in tomato;
- botrytis blight in lettuce and outdoor ornamentals; and
- seed rot caused by *Pythium* spp., *Rhizoctonia* spp. and *Fusarium* spp. on beans, peas, lima beans and soybean.

RootShield Biological Fungicides contribute to the suppression and management of plant diseases that might otherwise require frequent application of fungicides for disease control. The use of RootShield Biological Fungicides will help reduce fungicide use in greenhouses and the field, with consequent reduction in occupational, dietary and environmental exposure.

Measures to Minimize Risk

Labels of registered pesticide products include specific, legally enforced instructions for use. Directions include risk-reduction measures to protect human and environmental health. The key risk-reduction measures proposed on the labels of RootShield HC-Biological Fungicide Wettable Powder or RootShield Granules Biological Fungicide to address the potential risks identified in this assessment are as follows

Key Risk-Reduction Measures

Human Health

Because of concerns with users developing allergic reactions through repeated high exposures to *Trichoderma harzianum* Rifai strain KRL-AG2, anyone handling, mixing/loading, applying or involved in clean-up/repair activities of RootShield Biological Fungicides (wetable powder or granular formulation), must wear waterproof gloves, a long-sleeved shirt, long pants, eye goggles and a dust/mist filtering respirator (MSH/NIOSH approval number prefix TC-21C) or a NIOSH-approved respirator with any N-95, R-95, P-95 or HE filter. Furthermore, early-entry workers are restricted from entering areas where RootShield HC-Biological Fungicide Wettable Powder has been applied as a foliar spray for a period of four hours unless wearing the indicated personal protective equipment, with the exception of eye goggles and a dust/mist filtering respirator, which are required only until the spray mist has settled.

Environment

As a general precaution, label statements require handlers to not contaminate irrigation or drinking water or aquatic habitats. Instructions are also provided to limit runoff from fields treated with this product from entering lakes, streams, ponds or other water bodies. In addition, the label states that treated plant material must not be used as a substrate for mushrooms.

Next Steps

Before making a final registration decision on *Trichoderma harzianum* Rifai strain KRL-AG2, the PMRA will consider all comments received from the public in response to this consultation document. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications. The PMRA will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed final decision and the Agency's response to these comments.

Other Information

When the PMRA makes its registration decision, it will publish a Registration Decision on *Trichoderma harzianum* Rifai strain KRL-AG2 (based on the Science Evaluation of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

Science Evaluation

Trichoderma harzianum Rifai strain KRL-AG2

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active microorganism	<i>Trichoderma harzianum</i> Rifai strain KRL-AG2
Function	Biological Fungicide
Binomial name	<i>Trichoderma harzianum</i> Rifai strain KRL-AG2
Taxonomic designation	
Kingdom	Fungi
Phylum	Deuteromycotina
Order	Hyphomycetes (syn. Moniliales)
Genus	<i>Trichoderma</i>
Species	<i>harzianum</i>
Strain	KRL-AG2
Patent Status O.K. I information	None
Minimum purity of active	RootShield Technical contains a minimum of 5.0×10^8 colony forming units (CFU)/g dry weight of <i>Trichoderma harzianum</i> strain KRL-AG2 1.15% w/w (equivalent to a minimum of 10^7 CFU/g) in RootShield Granules biological Fungicide 1.15% w/w (equivalent to a minimum of 10^7 CFU/g) in RootShield HC-Biological Fungicide Wettable Powder

Identity of relevant impurities of toxicological, environmental and/or significance

The technical product does not contain any impurities or microcontaminants known to be Toxic Substances Management Policy Track 1 substances. A bacterial load of $>10^7$ CFU/g or the presence of a human pathogen will result in the rejection of a starter culture, termination of the production process or discarding of the final product. A stock culture is rejected if any fungal or bacterial contamination is found to be present. RootShield end-use products may contain antibiotic peptides collectively known as peptaibols. The absence of toxic effects in mammalian acute toxicity studies (see Section 3.0) suggests that the manufacturing process either does not favour the production of these potentially toxic metabolites or that the levels produced are too low to elicit an effect in animals administered a high dose of this fungus.

1.2 Physical and Chemical Properties of the Active Ingredients and End-Use Products

RootShield HC-Biological Fungicide Wettable Powder and RootShield Granules Biological Fungicide are manufactured as continuous process products. The technical grade active ingredient exists only transiently and is not packaged. Therefore, physical and chemical properties for the technical grade active ingredient have not been determined.

End Use Products—RootShield Granules Biological Fungicide and RootShield HC-Biological Fungicide Wettable Powder

Property	RootShield Granular Biological Fungicide	RootShield HC-Biological Fungicide Wettable Powder
Colour	Grey or green Standard Munsell 7.5Y 5/1.4	Grey or green Standard Munsell 2.5Y 8.3/2
Odour	Odourless to earthy	Odourless to earthy
Physical state at 25 °C	Coarse granular powder	Fine granular powder
Guarantee	Minimum of 1.0×10^7 CFU/g	Minimum of 1.0×10^7 CFU/g
Density	Bulk density: 0.61 g/cm ³ Tap density: 0.68 g/cm ³	Bulk density: 0.29 g/cm ³ Tap density: 0.63 g/cm ³

Identity of relevant impurities of toxicological, environmental and/or significance

The technical product does not contain any impurities or microcontaminants known to be Toxic Substances Management Policy Track 1 substances. A bacterial load of $>10^7$ CFU/g or the presence of a human pathogen will result in the rejection of a starter culture, termination of the production process or discarding of the final product. A stock culture is rejected if any fungal or bacterial contamination is found to be present. RootShield end-use products may contain antibiotic peptides collectively known as peptaibols. The absence of toxic effects in mammalian acute toxicity studies (see Section 3.0) suggests that the manufacturing process either does not favour the production of these potentially toxic metabolites or that the levels produced are too low to elicit an effect in animals administered a high dose of this fungus.

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End Use Products—RootShield Granules Biological Fungicide and RootShield HC-Biological Fungicide Wettable Powder

Property	RootShield Granular Biological Fungicide	RootShield HC-Biological Fungicide Wettable Powder
Colour	Grey or green Standard Munsell 7.5Y 5/1.4	Grey or green Standard Munsell 2.5Y 8.3/2
Odour	Odourless to earthy	Odourless to earthy
Physical state at 25°C	Coarse granular powder	Fine granular powder
Guarantee	Minimum of 1.0×10^7 CFU/g	Minimum of 1.0×10^7 CFU/g
Density	Bulk density: 0.61 g/cm^3 Tap density: 0.68 g/cm^3	Bulk density: 0.29 g/cm^3 Tap density: 0.63 g/cm^3

1.3 Directions for Use

RootShield HC-Biological Fungicide Wettable Powder and RootShield Granules Biological Fungicide are end-use products containing the active ingredient *Trichoderma harzianum* Rifai strain KRL-AG2. RootShield HC is sold as a wettable powder to be mixed with water and applied as a spray. RootShield Granules is a granular formulation that is mixed with soil or potting medium.

RootShield HC is to be used:

- as a foliar spray to suppress diseases caused by *Botrytis cinerea* in tomato, strawberry, lettuce and outdoor ornamental crops;
- as a drench application to suppress root diseases caused by *Pythium* spp., *Fusarium* spp. and *Rhizoctonia* spp.; and
- as a seed treatment for beans, lentil, pea, lima bean and soybean to suppress seedling root rot caused by *Pythium* spp., *Fusarium* spp. and *Rhizoctonia* spp.

Please refer to Regulatory Note REG2002-01, *RootShield Biological Fungicide, Trichoderma harzianum* Rifai strain KRL-AG2, for other uses of RootShield Biological Fungicide.

1.4 Mode of Action

Trichoderma is a genus of filamentous deuteromycetes that is ubiquitous in the environment. Its members are generally found in all soils including forest humus layer as well as agricultural and orchard soils. *Trichoderma* species are rarely reported to occur on living plants and have not been found as endophytes of living plants. *Trichoderma harzianum* is an antagonist of soil-borne fungal pathogens. Its mode of action is complex involving chemotaxis, antibiosis and parasitism. The initial interaction between the parasite and its host appears to be chemotropic growth. The hypha of the mycoparasite grows directly towards its host in response to secreted lectin(s). Apparently, lectins produced by the host bind to the galactose residues on *T. harzianum*'s cell wall and allow it to locate its prey. The antagonistic process starts in advance of physical contact. *Trichoderma harzianum* secretes a number of cell wall-degrading enzymes and antibiotics. These cell wall-degrading enzymes include β -1,3-glucanases, chitinases and proteinases. Several different volatile and non-volatile antibiotics such as diterpenes, peptaibols, butenolides, furanones, pyrones and pyridones, have been reported for *T. harzianum*. It is believed that these enzymes and antibiotics provide a synergistic effect on its host. It appears that the weakening of the host cell wall by the enzymes increases the rate of diffusion of the antibiotics through the cell wall. Upon physical contact, the hyphae of *Trichoderma harzianum* coil around its host where it proceeds with invasive growth. Shortly afterwards, the host hyphae collapse due to a loss of turgor pressure.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganism

Appropriate methodologies for detection, isolation and enumeration of the active ingredient, *Trichoderma harzianum* Rifai strain KRL-AG2, were previously detailed by the applicant. The microbial pest control agent (MPCA) had been fully characterized at the time of initial registration with respect to its origin of strain, natural occurrence and biological properties. No new information characterizing the MPCA was submitted by the applicant. The proposed amendment to the manufacturing process and expansion of use pattern is not expected to result in a change to this characterization.

The identification of *Trichoderma harzianum* to the species level is achieved using standard mycological techniques for this genus. Additional tests to distinguish strain KRL-AG2 from other *Trichoderma* species and strains of *Trichoderma harzianum* included isozyme electrophoresis and colony morphology on differential growth medium developed for strain recognition. The taxonomic position of strain KRL-AG2 was confirmed by microscopic examination of asexual reproductive structures (conidiospores and phialides) according to Rifai's species description.

Using starch gel electrophoresis, strain KRL-AG2 is differentiated from most other strains of *Trichoderma* by comparing 17 isozyme patterns to known allelic profiles. Known exceptions are *Trichoderma harzianum* strain T12 (and derivatives) and strain 1892, which express the same allele patterns as KRL-AG2 at the isozyme loci tested. An auxotrophic mutant of strain T12 was used in the production of strain KRL-AG2 by protoplast fusion techniques.

Trichoderma harzianum strain KRL-AG2 is also distinguished from other strains including strain T12 by colony morphology when propagated on a differential medium (CCNS, which is comprised of potato dextrose agar amended with cycloheximide, chlortetracycline, nystatin, streptomycin sulfate and Igepal). The CCNS cultures are incubated for 7 days at 25°C with 12-hour photoperiods. Colonies of KRL-AG2 are initially off-white in colour, producing little to no diffusible pigment in the surrounding agar medium. After several days of incubation, KRL-AG2 colonies produce green spore masses in diurnal zones of heavy sporulation, whereas other strains produce lighter colour spore masses with little diurnal variation in spore mass density.

The colony morphology of *Trichoderma harzianum* strain KRL-AG2 is influenced by conditions such as growth media and light. On potato dextrose agar, the perimeters of the colonies are white to cottony in appearance with green spores giving a pale to dark green colour to the centre of the colony. Under dense conditions the white aerial hyphae are less extensive giving rise to a denser spore mass.

No known toxic metabolites or hazardous substances are present in the RootShield HC-Biological Fungicide Wettable Powder and RootShield Granules Biological Fungicide end-use products. However, *Trichoderma harzianum* is a prolific producer of secondary metabolites, which include numerous types of alkyl pyrones, isonitriles, polyketides, oxygen heterocyclic compounds, diketopiperazines, and terpenoids (sesquiterpenes, diterpenes), which act either as antifungal/antibacterial agents or plant growth regulators. Of the many antibacterial and antifungal metabolites produced by *T. harzianum*, only a few present a potential risk to applicators and consumers of treated food crops. Of special interest is a unique class of linear hydrophobic polypeptides called peptaibols produced by most species and strains of *Trichoderma*, including *Trichoderma harzianum* KRL-AG2. Peptaibols function as antibiotics and contain a high proportion of α,α -dimethylisobutyric acid. Many peptaibols, such as the trichorzianines, trichokindins, trichorzins, trichorozins and harzianins, exhibit a broad range of bioactivities related to cell membrane perturbation.

2.2 Methods for Establishment of Purity of Seed Stock

Both the original mother culture and the starter cultures of *Trichoderma harzianum* Rifai strain KRL-AG2 are stored on grains of silica gel at -20°C to prevent genetic changes. Starter cultures are evaluated for genetic stability and contamination prior to use in production. Potential bacterial and fungal contaminants are monitored by standard microbiological methods. Potential relationships to known bacterial pathogens are determined. A stock culture is rejected if any fungal or bacterial contamination is present.

2.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products

Depending on the end-product formulation being manufactured, between three and five samples are taken from each lot during production and tested (CFU Test) using a standardized method to determine viability of the active ingredient. The CFU Test is performed to estimate the number of viable propagules of strain KRL-AG2 per unit mass of sample (CFU/g dry weight). The product guarantee is determined after the milling/deagglomeration and blending process and is expressed as CFU/g dry weight of product. No bioassays are performed to determine the potency of the final product against targeted seed pathogens.

2.4 Methods to Determine and Quantify Residues (Viable or Non-viable) of the Active Microorganism and Relevant Metabolites

Strains of *Trichoderma harzianum* are common in nature. The outdoor uses of strain KRL-AG2, in agricultural fields, nurseries, seeds and on turf to control fungal plant diseases is not expected to significantly increase natural environmental background levels of this microorganism. Furthermore, when *Trichoderma harzianum* strain KRL-AG2 was administered orally to rats, no signs of toxicity or disease were observed. Consequently, no methods are required to quantify viable or non-viable residues of *Trichoderma harzianum* strain KRL-AG2.

Although secondary metabolites of toxicological significance (peptaibols) have been shown to be produced by naturally occurring strains of *Trichoderma harzianum* (including strain KRL-AG2), use of RootShield Biological Fungicide products is not expected to result in a sustained increase in levels of these peptaibols beyond the naturally occurring background levels from *Trichoderma harzianum* native strains. Due to the proteinaceous nature of these polypeptides they are expected to be short lived in the environment once produced. They are susceptible to denaturing by ultraviolet light, high temperatures and various microbial processes in the environment. Furthermore, the results of supporting mammalian toxicity and pathogenicity studies did not indicate the presence of any metabolites of toxicological concern. No methodologies are required to quantify peptaibol residues.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality control procedures used to limit contaminating microorganisms during manufacture of RootShield Technical Grade Active Ingredient and RootShield Biological Fungicide end-use products are acceptable. Regular quality checks on the identity of *Trichoderma harzianum* are conducted during the production process by plating and by visual inspection to verify colony morphology and to detect any unusual colonies. The final product is also plated onto a number of selective media for detection of contaminating microorganisms. All bacterial and fungal colonies are purified and identified using traditional typing methods as well as molecular "genetic fingerprint" analysis when necessary. A bacterial load of $>10^7$ CFU/g or the presence of a human pathogen will result in rejection of a starter culture, termination of the production process or discarding the final product.

2.6 Methods to Show Absence of Any Human and Mammalian Pathogens

As noted in Section 2.5, several approaches are used to limit microbial contamination in RootShield Biological Fungicide products. These procedures include frequent purity checks to detect any unusual colonies and to verify colony morphology, as well as standard assays designed to detect contaminating fungi and total aerobes, yeasts and *Aspergillus* species, coliform bacteria, *Staphylococcus*, *Streptococcus* and *Salmonella*.

Acceptable microbial contaminant analysis data were submitted for five batches of the RootShield HC-Biological Fungicide Wettable Powder end-use product.

2.7 Methods to Determine Storage Stability, Shelf-life of the Microorganism

The viability of *Trichoderma harzianum* strain KRL-AG2 in RootShield end-use products (wettable powder and granular formulations) was previously assessed by determining the guarantee (as described in Section 2.3) over a period of time and over a range of storage temperatures. No new storage stability data were submitted.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

Naturally occurring spores of *Trichoderma harzianum* can be found in the soil. Furthermore, infection by *Trichoderma harzianum* strain KRL-AG2 in animals is not likely as the MPCA does not reportedly grow at temperatures above 32°C.

A detailed review of the toxicological database for *Trichoderma harzianum* strain KRL-AG2 has been completed. The database for *Trichoderma harzianum* strain KRL-AG2 is complete (see Appendix I) consisting of laboratory animal (in vivo) toxicity studies (acute oral toxicity, acute pulmonary toxicity and infectivity, acute intravenous injection infectivity, acute inhalation toxicity, acute dermal toxicity, and dermal irritation) currently required for health hazard assessment purposes. These were carried out in accordance with currently accepted international testing protocols and Good Laboratory Practices.

The scientific quality of the data is high and the database is considered sufficient to characterize the toxicity and infectivity of this MPCA and the two end-use products.

In an acute oral toxicity/pathogenicity study, there were no mortalities, no changes in body weight and no necropsy findings in Sprague Dawley rats (13/sex) following administration of a single oral dose of approximately 10^8 CFU *Trichoderma harzianum* strain KRL-AG2. A distinct and rapid pattern of clearance of the test microbe by posttreatment day 2 was demonstrated. Based on the results of this study, strain KRL-AG2 is of low toxicity in the rat when challenged via the oral route.

In an acute pulmonary toxicity and infectivity study, there were no mortalities, no changes in body weight gain and no treatment-related necropsy findings observed in Sprague Dawley rats (15/sex) following a single intratracheal instillation of approximately 10^8 CFU of the test organism. An equal number of untreated control rats were maintained for the same 21-day test period as the treated animals. Treatment-related respiratory tract lesions consisting of mottled or enlarged and mottled lungs in animals of both sexes were observed at scheduled necropsy for up to three weeks following dosing. Necropsy revealed mottled lungs in 11 treated males and females, and enlargement of the lungs in six treated rats of both sexes. These pulmonary lesions were most likely the remnants of the inflammation that would have accompanied the initial instillation and "clean-up" of destroyed fungal cells by immune and phagocytic cells. Following instillation, the test organism was detected predominantly in the lungs of treated animals on day 1 with clearance of *Trichoderma harzianum* by study termination at day 21. Results of the infectivity/clearance portion of the pulmonary study demonstrated that the immune system was intact and able to process and clear the active ingredient from the body by study termination. Furthermore, all treated animals were surveyed to scheduled termination and all appeared normal for the duration of the study—except on the day of dosing when they all appeared lethargic. No control animals showed any unusual necropsy findings. This toxicity and pathogenicity testing of *Trichoderma harzianum* strain KRL-AG2 in male and female Sprague Dawley rats was classified

as acceptable. Based on the results of this study, there was no evidence of treatment-related toxicity or infectivity related to a single intratracheal instillation of *Trichoderma harzianum* strain KRL-AG2 at a dose of approximately 10^8 CFU per test animal.

In an acute intravenous infectivity study, no mortalities and no significant toxicity were observed in Sprague Dawley rats (15/sex) following intravenous injection of approximately 10^7 CFU of the test organism. An equal number of untreated control rats were maintained for the same 21-day test period as the treated animals. Treatment-related effects were limited to an enlargement of the spleen in male and female treated animals. This was considered to be a normal reaction to microbial antigens. Following injection, the test microbe was predominantly found in the liver, lungs, spleen, kidney and blood of treated animals. Enlarged spleens were observed in nine of the treated animals. A drastic reduction in number of test organisms and a distinct pattern of clearance of the test microbe were demonstrated by post-treatment day 21. This toxicity and pathogenicity testing of *Trichoderma harzianum* strain KRL-AG2 in males and female Sprague Dawley rats was classified as acceptable. Based on the results of this study, there was no evidence of toxicity or pathogenicity related to treatment by intravenous injection with *Trichoderma harzianum* strain KRL-AG2 at a dose of approximately 10^7 CFU per test animal.

Rationale previously submitted to waive the requirements for both the dermal toxicity and the primary dermal irritation studies for RootShield HC-Biological Fungicide Wettable Powder, previously RootShield Drench, and RootShield Granules Biological Fungicide was acceptable based on the following factors:

- the absence of adverse effect reports for workers involved in their manufacture and use of end-use products
- the active microorganism's low-toxicity and no-pathogenicity rankings in acute oral, pulmonary and intravenous tests
- the minor toxicological concern and widespread commercial use of the formulation inert (formulants)

The eye irritation potential of *Trichoderma harzianum* strain KRL-AG2 was tested by observing the effects following a single dose of approximately 10^8 CFU of the test organism to one eye in each of six New Zealand White rabbits. Slight redness in the conjunctivae was observed in all treated eyes at the one-hour observation period. All eyes appeared clinically normal by 72 hours and no other signs of irritation were observed during the seven-day observation period. The maximum irritation score reported for the study was 2.3/110 (after one hour). This primary eye irritation testing of *Trichoderma harzianum* strain KRL-AG2 in New Zealand White rabbits was classified as acceptable. Based on the results of this study, *Trichoderma harzianum* strain KRL-AG2 was determined to be minimally irritating at a dose of approximately 10^8 CFU per rabbit eye and did not require signal words on the product labels. However, the test substance was a pure powder preparation of strain KRL-AG2 and the end-use products contain formulants that are eye irritants.

A skin sensitization study was not submitted on the microbial *Trichoderma harzianum* strain KRL-AG2 as the PMRA does not require hypersensitivity studies to support the registration of microbial pest control agents. During product research and development activities as well as operational applications in the United States, where the active ingredient has been registered since 1990, individuals would have been exposed to both the mycelia and spores of the MPCA. Exposure is likely to have occurred via dermal and inhalation routes. Neither report nor suggestion of hypersensitivity to this fungus has been noted during this time. Nevertheless, because most microorganisms contain substances that elicit positive hypersensitivity reactions in humans, *Trichoderma harzianum* strain KRL-AG2 is considered to be a potential sensitizing agent. Consequently the signal words **POTENTIAL SENSITIZER** are required on the principal display panels of RootShield technical and end-use formulation labels.

Higher tier subchronic and chronic toxicity studies were not required because of the low acute toxicity of the MPCA, and there were no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral toxicity, pulmonary toxicity/infectivity and intravenous injection infectivity.

The active ingredient *Trichoderma harzianum* strain KRL-AG2 is not known to be a human pathogen or an endocrine disruptor. The available scientific literature provides no reports that suggest *Trichoderma harzianum* has the potential to cause adverse effects on the endocrine system of animals. The submitted toxicity/infectivity studies in the rodent indicate that, following pulmonary and intravenous injection routes of exposure, the immune system is still intact and able to process and clear the MPCA. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for *Trichoderma harzianum* strain KRL-AG2.

3.2 Occupational and Bystander Exposure and Risk Assessment

3.2.1 Occupational

When handled according to label instructions, the potential for dermal, eye and inhalation exposure for applicators, mixer/loaders, and handlers exists, with the primary source of exposure to workers being dermal. Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *Trichoderma harzianum* has not been identified as a dermal wound pathogen, and there is no indication that it could penetrate intact skin of healthy individuals. Also, infection in animals is unlikely given that the MPCA does not grow at temperatures above 32°C. Furthermore, growth of *Trichoderma harzianum* strain KRL-AG2 is host specific; no toxicity and no irritation is expected from strain KRL-AG2 via the dermal exposure route. Toxicity testing with *Trichoderma harzianum* strain KRL-AG2 also showed no signs of toxicity or infectivity via the oral, pulmonary or intravenous injection routes of exposure.

The PMRA assumes all microorganisms contain substances that can elicit positive hypersensitivity reactions, regardless of the outcome of sensitization testing. Label statements (Potential Sensitizer, for example) and risk mitigation measures such as personal protective equipment, including gloves, long-sleeved shirts, long pants, NIOSH approved respirators (with any N-95, P-95, R-95 or HE filter), shoes and socks, are required to minimize exposure and protect handlers who are likely to be primarily exposed.

3.2.2 Bystander

Inhalation or dermal exposure to the general public is expected to be low from the proposed foliar application of RootShield HC-Biological Fungicide Wettable Powder to agricultural crops and nursery stock. Overall, the PMRA does not expect that bystander exposures will pose an undue risk on the basis of the low toxicity/pathogenicity profile for *Trichoderma harzianum* strain KRL-AG2 and the related end-use products.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

Even though *Trichoderma harzianum* is a ubiquitous organism found in most terrestrial environments, *Trichoderma* species are rarely reported to occur on living plants. The proposed outdoor use pattern as a foliar spray to food crops is likely to result in only low levels of residues on treated food commodities at the time of harvest. This MPCA or any secondary metabolites it may produce do not persist when applied to foliage or fruit due to ordinary environmental conditions (for example, ultraviolet light).

Furthermore, any residues of the active microorganism are likely to be removed from treated food by washing, peeling, cooking and processing. Even if residues are not removed, dietary exposure to the microbial agent is unlikely to result in any undue hazard to consumers because no adverse effects were observed at maximum hazard dose levels in the submitted Tier I acute oral study.

Dietary exposure to secondary metabolites (peptaibols) produced by strain KRL-AG2 may be possible from consumption of foliage or fruits of food commodities treated with *Trichoderma harzianum* strain KRL-AG2. However, the level of these peptaibols remaining on leaf or fruit surfaces is expected to be low as the peptaibols are proteinaceous in nature and easily denatured by ultraviolet light, low humidity and other microbial processes present in the environment. In addition, these residues may be removed by washing, peeling or processing of commodities. Uptake of fungal metabolites by plant roots and translocation to fruit is possible for metabolites produced by the actively growing fungus in soil treated with RootShield Biological Fungicide products, but no crop residue data were submitted for any of the secondary metabolites that may present a human health concern, specifically peptaibols antibiotics. However analytical data on the peptaibol production levels from strain KRL-AG2 relative to at least one other naturally occurring strain of *Trichoderma harzianum* suggest the MPCA produces peptaibols at levels

unlikely to exceed those produced by naturally occurring isolates in the environment. Furthermore, given the long history of safe use of products containing this active ingredient in the United States as indicated by the absence of adverse effects reports, residue levels of these metabolites are likely to be sufficiently low in the crop at the time of harvest so as to not provoke concern for dietary exposure.

Higher tier subchronic and chronic dietary exposure studies were not required because of the low toxicity of the MPCA and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral toxicity and acute pulmonary and intravenous, injection toxicity/infectivity studies. Therefore, there are no concerns for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children.

3.3.2 Drinking Water

The likelihood that *Trichoderma harzianum* strain KRL-AG2 could enter neighbouring aquatic environments via spray drift or surface water runoff from field use of RootShield Biological Fungicide (granular or powder formulations) is considered low. *Trichoderma harzianum* strain KRL-AG2 is not generally recognized as an aquatic microorganism and, therefore, is not expected to proliferate in aquatic habitats following direct or indirect application to bodies of water—such as runoff from treated agricultural fields and turf. Moreover, *Trichoderma harzianum* is not considered to be a risk to drinking water sources. Accordingly, drinking water is not specifically screened for *Trichoderma harzianum* as a potential indicator of microbial contamination or as a direct pathogenic contaminant. Both percolation through soil and municipal treatment of drinking water would reduce the possibility of significant transfer of residues to drinking water. Also, the labels for RootShield Biological Fungicide (granules and wettable powder formulations) will instruct users not to contaminate drinking water supplies or aquatic habitats through equipment cleaning or waste disposal. Users must not allow effluent or runoff from greenhouses containing this product to enter lakes, streams, ponds or other waters. Therefore the potential of exposure and risk via drinking water is likely to be minimal to non-existent for this MPCA.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculations of acute reference doses and acceptable daily intakes are not usually possible for predicting acute and long-term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the PMRA concludes that *Trichoderma harzianum* strain KRL-AG2 is of low toxicity, not pathogenic or infective to mammals, and infants and children are likely to be no more sensitive to the MPCA than the general population. Thus, there are no threshold effects of concern and, as a result, no need to require definitive, multiple-dose testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of

exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of the MPCA, including neurological effects from pre- or postnatal exposures, and cumulative effects on infants and children of the MPCA and other registered micro-organisms that have a common mechanism of toxicity does not apply to this MPCA. As a result, the PMRA has not used a margin of exposure (safety) approach to assess the risks of *Trichoderma harzianum* strain KRL-AG2 to human health.

3.4 Maximum Residue Limits

The *Food and Drugs Act* prohibits the sale of adulterated food, that is, food containing a pesticide residue that exceeds the established MRL. Pesticide MRLs are established for *Food and Drugs Act* purposes through the evaluation of scientific data under the *Pest Control Products Act*. Each MRL value defines the maximum concentration in parts per million (ppm) of a pesticide allowed in or on certain foods. Food containing a pesticide residue that does not exceed the established MRL does not pose an unacceptable health risk.

The use of RootShield HC-Biological Fungicide Wettable Powder and RootShield Granules Biological Fungicide is not expected to result in a sustained increase in background levels of this organism or the secondary metabolites it could potentially produce. The secondary metabolites are expected to have a short residency time in the environment. As such, the level of anticipated exposure to any potential secondary metabolites produced by the MPCA or residues of the MPCA itself is extremely low. No adverse effects from dietary exposure have been attributed to natural populations of *T. harzianum*, and no adverse effects were observed in the acute oral toxicity study with *Trichoderma harzianum* strain KRL-AG2. Furthermore, there have been no reports of adverse effects to humans from natural populations of *T. harzianum*. Therefore, the establishment of an MRL is not required for *Trichoderma harzianum* strain KRL-AG2 under section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, section B.15.002 of the Food and Drugs Regulations.

3.5 Aggregate Exposure

Based on the toxicity and infectivity test data submitted and other relevant information in the PMRA's files, there is reasonable certainty no harm will result from aggregate exposure to residues of *Trichoderma harzianum* strain KRL-AG2 to the general Canadian population, including infants and children, when the microbial pest control product is used outdoors as labelled. This includes all anticipated dietary exposures (through food and drinking water) and all other non-occupational exposures (through dermal or inhalation) for which there is reliable information. Furthermore, there have been no adverse effects from exposure to natural populations of *Trichoderma harzianum* in the environment. Even if there is an increase in exposure to this microorganism from outdoor uses of RootShield Biological Fungicide products, there should not be any increase in potential human health risk.

3.6 Cumulative Effects

The PMRA has considered available information on the cumulative effects of such residues and other substances that have a common mechanism of toxicity, including the effects on infants and children. Besides naturally occurring strains of *Trichoderma harzianum* in the environment, the PMRA is not aware of any other microorganisms, or other substances, that share a common mechanism of toxicity with this active ingredient. No cumulative effects are anticipated if the residues of *Trichoderma harzianum* strain KRL-AG2 interact with related strains of this microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

Environmental fate testing is intended to demonstrate whether a MPCA is capable of surviving or replicating in the environment to which it is applied. The testing could also provide an indication of which non-target organisms may be exposed to the MPCA as well as provide indications to the extent of exposure. Environmental fate data (Tier II/III) are not normally required at Tier I and are only triggered if significant toxicological effects in non-target organisms are noted in Tier I testing.

For the initial decision to register RootShield end-use products for greenhouse uses, information on the fate and behaviour of *Trichoderma harzianum* strain KRL-AG2 in the environment was submitted by the applicant and reviewed by the PMRA.

Trichoderma species are ubiquitous soil-dwellers, inhabiting soil, rotting wood and vegetable matter in virtually all terrestrial environments. They produce copious conidia held together in mucoid spore balls, which can be disseminated by water and by soil fauna such as insects and earthworms. With respect to its abundance relative to other species of *Trichoderma*, *Trichoderma harzianum* has been described as more characteristic of warm climates; however, it is evident from the field study and published literature that cold-tolerant strains do exist. It is also evident that *Trichoderma harzianum* KRL-AG2 will likely disseminate and persist in the Canadian environment following its release. The applicant previously submitted a study report that evaluated *Trichoderma harzianum* strain KRL-AG2's ability to survive over the winter and to colonize subsequent crops. *Trichoderma harzianum* strain KRL-AG2 was detected in soil collected from the microplots in the spring of 1989 and thus survived over the winter. Its isolation from soils collected from untreated crops suggested that the active ingredient was rapidly disseminated in soil. However, no comparisons could be made between treated and untreated groups. Surviving populations of *Trichoderma harzianum* strain KRL-AG2 were shown to colonize roots of subsequent crops. Adverse effects, however, are not expected as *Trichoderma harzianum* is a saprophyte, with the exception that it can attack other fungi. Furthermore, *Trichoderma harzianum* strain KRL-AG2 has been used in the United States for a number of years with no reports of adverse environmental effects.

Based on the information submitted previously, this MPCA does not persist when applied to foliage or fruit. Ordinary environmental conditions cause rapidly declining population levels of the microbe soon after application to above-ground plant parts. The proposed outdoor application of RootShield Biological Fungicide products is not expected to result in a sustained increase of populations of the MPCA beyond those of naturally occurring soil dwelling *Trichoderma* species.

4.2 Effects on Non-Target Species

Previously submitted waiver rationales were used to address most of the environmental toxicology requirements. These rationales were based on the ubiquitous nature of *T. harzianum*, the lack of reported adverse effects in the literature, the inability of *Trichoderma harzianum* to become established in unpolluted aquatic environments and the limited persistence expected of secondary metabolites that may be produced.

4.2.1 Effects on Terrestrial Organisms

The applicant previously submitted a study considered as supplemental, which assessed the acute oral toxicity of *Trichoderma harzianum* strain KRL-AG2 to 21-day-old Bobwhite quail (*Colinus virginianus*) over 30 days. No treatment-related effects or mortalities were observed in any of the quail treated with live *Trichoderma harzianum* strain KRL-AG2, attenuated *Trichoderma harzianum* strain KRL-AG2 or sterile filtrate broth. The LD₅₀ was determined to be greater than 11 110 mg a.i./kg bw (or approximately 4×10^9 CFU/kg bw), however, insufficient information and data were provided to determine if the dose received by the birds was viable. No new data were submitted.

There is the potential for oral exposure to birds from outdoor uses, as birds may consume food items that might have been exposed to *Trichoderma harzianum* strain KRL-AG2 (for example: treated seeds, non-target insects and other plant material). Risk is expected to be minimal, however, based on the lack of demonstrated toxicity and or pathogenicity via the oral route in birds and rats, the ubiquitous nature of *Trichoderma harzianum* and lack of reported adverse effects in published scientific literature. Furthermore, *Trichoderma harzianum* strain KRL-AG2 is not a known pathogen of birds, and avian body temperatures are not conducive to the growth of the MPCA.

The applicant previously submitted a request to waive the requirement for an acute inhalation toxicity study for birds, based on the lack of adverse effects noted in the avian oral study and in the rat acute pulmonary study. No adverse effects were noted in either study. However, the acute avian oral study was judged supplemental due to lack of data. In addition, birds tend to be more sensitive to effects through inhalation than mammals because of the air sacs throughout their body. Consequently, the extrapolation of the avian oral toxicity or the rat pulmonary study to possible avian pulmonary effects is not possible. No new avian data were submitted. Based on the proposed outdoor use pattern, the potential for exposure through inhalation to birds does exist, although minimally. However, given the ubiquitous nature of *Trichoderma harzianum* and

lack of reported adverse effects in published scientific literature, there would be little concern for inhalation exposure to birds. Furthermore *Trichoderma harzianum* strain KRL-AG2 is not a known pathogen of birds, and avian body temperatures are not conducive to the growth of the MPCA.

A waiver rationale was previously submitted for terrestrial arthropod and non-arthropod invertebrate testing, based on the absence of published scientific literature in which *Trichoderma harzianum* caused infection or any other impact on insects or other invertebrates. In one published study, bees were used to disseminate *Trichoderma harzianum* strain KRL-AG2 without any apparent adverse effects. In another, no adverse effects were noted for hives treated with *Trichoderma harzianum* strain T-39. Rather, literature indicated that insects, especially mites, consumed the hyphae of *Trichoderma* species. While terrestrial arthropods and other invertebrates may be exposed from the proposed outdoor uses of RootShield Biological Fungicide products, the risk to these organisms is considered low based on the lack of adverse effects in published scientific literature.

A waiver rationale was previously submitted for microorganism testing for the initial registration, supported by a search of published scientific literature. Aggressive strains of *Trichoderma harzianum* were identified in published literature as the cause of "green mold disease," resulting in significant economic losses in the commercial mushroom industries in Europe and North America. A concern was identified from the use of plant material treated with RootShield Biological Fungicide products, as a growing substrate in mushroom houses. As a result, a mitigative statement was required on the label prohibiting the use of treated material in mushroom growing facilities.

Given that *Trichoderma* species are ubiquitous and considering the above information, the risk to beneficial microorganisms is considered to be low from the proposed outdoor use pattern for RootShield Biological Fungicide products (wetable powder and granular formulations).

A waiver rationale was previously submitted for terrestrial plants supported with results of a search of published literature. *Trichoderma harzianum* is a ubiquitous fungus, but it is rarely reported to occur on living plants and is not an endophyte. Despite the potent cellulolytic enzyme systems and secondary metabolites with plant growth regulating properties, the ability of *Trichoderma harzianum* to attack living wood or plants is considered weak. Although the potential exists for non-target plants to be exposed to strain KRL-AG2 through spray drift when applied outdoors, based on the lack of adverse effects in the scientific literature, risk is expected to be minimal if the product is applied as directed on the label.

The risk to terrestrial organisms from exposure to the secondary metabolites (peptaibols) of *Trichoderma harzianum* strain KRL-AG2 is also expected to be minimal. The lack of adverse effects from the registered outdoor uses of this MPCA in the United States suggests the exposure of non target terrestrial organisms to these secondary metabolites are not at toxicologically significant levels. Peptaibols that may be produced by this and other strains of *Trichoderma harzianum* are not expected to persist, as they are easily denatured by ultraviolet light, heat and

various microbial processes in the environment. Based on all the available data and information on the effects of *Trichoderma harzianum* strain KRL-AG2 to terrestrial organisms, there is reasonable certainty no harm will be caused to birds, wild mammals, arthropods, non-arthropod invertebrates, plants or to other non-target microorganisms from the proposed outdoor use of the biological fungicides, RootShield HC-Biological Fungicide Wettable Powder and RootShield Granules Biological Fungicide.

4.2.2 Effects on Aquatic Organisms

Rationales were previously submitted to waive studies addressing the potential risk RootShield Biological Fungicide products may pose to the aquatic environment. No new data were submitted. *Trichoderma harzianum* is a ubiquitous soil dweller that produces copious conidia held together in mucoid spore balls, which can be disseminated in water. Based on the proposed outdoor use pattern, exposure to aquatic environments may occur from runoff events of treated agricultural fields and golf courses or from spray drift during foliar applications. Few references, however, have cited instances of recovery of *Trichoderma* from fresh or salt water environments unless the water was polluted. The lack of evidence on the natural occurrence of *Trichoderma harzianum* in freshwater or salt water indicates a failure to establish itself in these environments, and the negligible impact of introduced inoculum on the ecologies of freshwater and salt water habitats.

A search of published scientific literature found one reference in which eastern oyster (*Croostrea gigas*) embryos exposed to sediment extracts displayed developmental effects (a protruding mantle) by the D-larval stage. Analysis of the extracts implicated peptaibols (metabolites produced by many strains of *T. harzianum*, including strain KRL-AG2) in the disruption of embryogenesis, but these were not thought to be solely responsible. In the same article, *Trichoderma longibrachiatum* was isolated from the blue oyster and cultured. Peptaibols, extracted and identified from cultures of the fungus, elicited embryotoxic effects in assays performed on oyster embryos. Although *Trichoderma harzianum* strain KRL-AG2 is known to produce similar peptaibols as those investigated in the study, *Trichoderma harzianum* strain KRL-AG2 is not expected to establish itself in aquatic environments in the event of runoff or spray drift from treated fields. Therefore, sustained levels of these peptaibols or the MPCA itself, beyond that of naturally occurring *Trichoderma* strains, are not expected from the proposed outdoor uses. Furthermore, standard label statements required for agricultural pesticides would further reduce the potential for exposure to aquatic environments.

Based on all the available data and information on the effects of *Trichoderma harzianum* strain KRL-AG2 to aquatic organisms, there is reasonable certainty no harm will be caused to non-target aquatic organisms from the proposed outdoor uses of RootShield biological fungicides. As a precaution, standard label statements will prohibit handlers from contaminating aquatic habitats, provide instructions on preventing runoff from treated fields and not allow effluent from greenhouses containing this product from to enter streams, ponds or other water bodies.

5.0 Value

5.1 Effectiveness Against Pests

The efficacy data package submitted in support of the claims for suppression for the various diseases consisted of 26 trials (three on ornamentals, four on strawberry, three on tomato, six on lettuce, two on turf and eight on seed treatment of pulse crops).

5.1.1 Acceptable Efficacy Claims

5.1.1.1 Acceptable Efficacy Claims for RootShield HC-Biological Fungicide Wettable Powder Foliar Application

5.1.1.1.1 Suppression of *Botrytis Cinerea*

Grey Mould on Strawberry

Results from four field trials with low disease pressure were submitted. Of these, three were conducted in New York and one was conducted in Florida. Two of the New York studies tested applications of RootShield by conventional ground equipment as well as bees. The rate used in the trials (10 g/L) was higher than the proposed rate (3.5–7.5 g/L). When the trials applied RootShield as a foliar spray once—either at early or late bloom stage—disease incidence was significantly reduced by 32–72% (with a mean of 49%) under low disease pressure compared to untreated controls. Generally, early bloom applications resulted in better disease control compared to late bloom applications. One trial, with two applications of RootShield, resulted in a 71% reduction in diseased berries compared to a 51% reduction when RootShield was applied at the late bloom stage. Bee delivery of RootShield resulted in a better disease control compared to foliar applications, indicating a more targeted application, which ensures RootShield reaches the infection court, will result in better control of the disease. In trials in which RootShield was compared to a commercial standard, Ronilan (active ingredient: vinclozolin) performed better in one of two trials. The claim for suppression of botrytis grey mold in strawberry is supported at 10 g/L based on the efficacy data provided.

Botrytis Stem Canker on Tomato

Three greenhouse trials were conducted on tomato. In one trial, there was low disease pressure. In the two other studies, it was difficult to ascertain disease pressure in the trials since the parameter used to assess disease was lesion length. Disease pressure is assumed to be moderate. The rate used in the trials (0.6 g/L) was considerably less than the proposed rate range (3.75–7.0 g/L).

In the first trial, RootShield reduced disease severity by 77% compared to the untreated check. The commercial standard, chlorothalonil, reduced the disease by only 29%. RootShield reduced disease severity on tomato fruits by 23%, but this level of control was not significantly different from the untreated check.

When *Trichoderma harzianum* was applied at 0.6 g/L one to four times, lesion length was reduced anywhere from 6 to 80%. When six applications were made, lesion length was reduced more consistently between 62 and 86% (mean: 71%). Fruit yield/plant significantly increased by 56% in treated plots in 1998 but not 1999. The performance of *Trichoderma harzianum* was better than the commercial standard, iprodione, in the 1998 trial. Considering the tested rate (0.6 g/L) reduced the disease, it is expected the proposed rate of 3.75 g/L will suppress the disease as well. The proposed high rate of 7.5 g/L is not supported because this rate was not tested in the trials and no justification was provided for its use on tomato. The data support the claim for suppression of *Botrytis* stem canker in greenhouse tomato. This claim is extended to field tomato because disease development is the same. In addition, it is expected that the disease will be suppressed under field conditions at the proposed rate of 3.75 g/L based on the lower rate (0.6 g/L) tested in greenhouse trials. The proposed higher rate of 7.5 g/L is not supported.

Botrytis Blight on Lettuce

A total of six greenhouse trials were conducted in New York on lettuce, but they were presented as two sets of data that consisted of averaged results from three trials each. One set of three trials tested a preventative application of RootShield while the other set tested a curative application. Disease pressure was moderate to high. Efficacy of RootShield was evaluated using the Relative Plant Health parameter and consisted of ratings from 0 to 5 (dead to healthy plants, respectively). No detailed description of the rating scale was provided. The trials tested a rate of 10 g/L, which is higher than the proposed rate of 3.75–7.5 g/L. It appears that only one application was made.

A curative application of RootShield resulted in a plant rating of 3.4 compared to 1.4 for untreated plants, indicating that plants sprayed with RootShield were healthier than the untreated control. The effectiveness of RootShield as a curative treatment increased when it was applied with a surfactant, resulting in a plant rating of 4.1. The performance of RootShield + R-11 was comparable to or better than Chipco (fosetyl AL). However, Chipco is not considered a commercial standard since it is not registered on lettuce in Canada. The same trend was observed in the trials with the preventative application of RootShield. The claim for suppression of botrytis blight on lettuce is supported at 10 g/L based on the efficacy data provided.

Botrytis Blight on Ornamentals

Two field trials on geranium conducted in New York and Oregon were provided to support the claim of suppression of *Botrytis* on outdoor nursery crops. It is not known whether the test plants were *Geranium* spp. (perennial geranium) or *Pelargonium* spp. (annual geranium).

In the New York trial, RootShield was applied before the plants were inoculated with *Botrytis cinerea*. The applicant stated that a rate of 7.5 g/L was used as per the American label. Disease pressure could not be determined. The number of leaves with *Botrytis* lesions differed significantly between the untreated check and RootShield-treated plants. A foliar spray of RootShield provided 54% disease reduction. In this trial, the performance of RootShield was comparable to Chipco (contains fosetyl AL). Chipco is not registered for *Botrytis* control for ornamentals in Canada, however, so it cannot be considered a commercial standard. In the Oregon trial, disease pressure was low to moderate. The data showed that one preventative

application of RootShield at 7.5 g/L significantly reduced the disease compared to the untreated check. Disease ratings on the leaves of plants treated with the high rate of RootShield were comparable to healthy plants not inoculated with *Botrytis cinerea* (0.72 and 0.99, respectively). However, when plants were treated with the lower rate of 2.5 g/L they still showed disease ratings similar to the untreated inoculated control group.

The results support the claim of suppression of botrytis blight caused by *Botrytis cinerea* on geranium at the rate of 7.5 g/L. There is no data that demonstrate that the rate of 3.5 g/L will result in suppression of the same disease. The claim for suppression of *Botrytis cinerea* on outdoor nursery crops is supported based on extrapolation from the use on geranium. The trials on tomatoes, strawberry and lettuce also provide additional evidence of the efficacy of RootShield on *B. cinerea*.

5.1.1.1.2 Suppression of *Pythium* spp., *Rhizoctonia* spp. and *Fusarium* spp.

5.1.1.1.2.1 Seed Treatment

Eight seed treatment trials—two on lima beans, one on peas, four on dry beans and one on soybean—were submitted to support a claim of suppression of root diseases caused by *Pythium*, *Rhizoctonia* and *Fusarium*.

The studies on lima beans tested rates similar to the proposed rates of 60 and 125 g/50 kg seed. Emergence significantly increased by 8 to 24% over the untreated checks at 60g/50 kg seed and by 12 to 19% at 120 g/50 kg seed. There was no significant difference in performance between the lower and higher rates, but it is expected the higher rate will reduce disease further under conditions of high disease pressure. The performance of the commercial standards, Apron XL (containing metalaxyl) and Maxim (containing fludioxonil), were the same as or better than RootShield.

A study on peas demonstrated that considerably more plants emerged where seeds have been treated with RootShield at approximately 60g/50 kg seed. However, root rot ratings were not significantly different between the treated seeds and the untreated check. Area under plant height progress curve (AUPHPC) values were not significantly different between RootShield and the untreated controls. The field was not inoculated with any specific pathogen in this trial but it had a strong history of pea root disease and the researchers stated that *Aphanomyces*, *Fusarium*, *Phoma*, and *Pythium* have been isolated from diseased peas planted in this site in the past. When soybean seeds were treated at a rate of 28 g RootShield/50 kg seed, plant stand counts and root lesion lengths were not significantly different from the control. The performance of RootShield was not significantly different from the commercial standard, Apron Maxx RTA.

The effect of RootShield on root rot in dry beans was inconsistent. In two trials in Nebraska, where a rate slightly higher than the proposed rate was used (equivalent to 136 g/50 kg seed), a significant difference was noted in root rot ratings in one trial but not in the other. Yields were not significantly different between RootShield treated seeds and the control seeds in both trials.

Two additional dry bean studies from North Dakota conducted in 1990 were submitted, but the rate used was stated as 34 mL of *T. harzianum*/kg seed. No other details about the rate were provided so it is difficult to compare the tested rates to the proposed rates. The results show that root rot ratings and plant stand counts were significantly different between RootShield and the control in one trial but not in the other. The claim for suppression of root rot is not supported by the trials because:

- the data provided for the lima bean trials are not direct assessments of root disease;
- the results of the dry bean trials were inconsistent; and
- root rot was not reduced by RootShield in the pea and soybean trials.

However, the lima bean, pea and bean trials did demonstrate that RootShield improved plant emergence. The trials relied on natural inoculum in fields with a history of seedling diseases caused by *Fusarium* spp., *Rhizoctonia* spp. and *Pythium* spp. Plant stand counts are used as an assessment parameter for seed rot caused by various seed and soil-borne pathogens. Therefore, the claim for suppression of seed rot caused by *Fusarium*, *Pythium* and *Rhizoctonia* on pea and lima bean is supported based on the efficacy data provided as well as the currently registered use pattern that indicates RootShield suppresses *Fusarium* spp., *Rhizoctonia* spp. and *Pythium* spp. This claim is extrapolated to soybean and lentil based on similar disease development. The use of RootShield on chickpeas is not supported at this time since root rot, a disease on chickpea, is caused by *T. harzianum*. If the applicant wishes to include this crop on the label, additional trials are required to determine that the strain of *Trichoderma harzianum* contained in RootShield will not infect chickpeas. Extrapolation of the claim on root rot to all crops grown from seed is not supported.

5.1.1.1.2.2 Drench Application

Root diseases on greenhouse peppers

No data were submitted for this claim. The product is currently registered as a soil drench for tomatoes at the same rate. The use on peppers can be supported since peppers are considered similar to tomatoes in cropping practice. Disease development on both crops is also similar. The rationale supports a soil drench claim for suppression of root rot caused by *Pythium* spp., *Rhizoctonia* spp. and *Fusarium* spp. on peppers.

Root diseases on outdoor ornamental crops

Three greenhouse trials (one on geranium, one on pansy and one on poinsettia) were submitted to support the claim for suppression of root diseases on outdoor ornamental crops. The trials on pansy and poinsettia are considered supplementary because no direct assessments of root disease were made; performance assessments consisted of descriptions of root quality. In addition, poinsettia is grown exclusively in greenhouses in Canada and are not representative of outdoor ornamental crops. Both pansy and the species of geranium used in this trial are annual plants typically grown in greenhouses in Canada prior to being sold as bedding plants, so they are not representative of outdoor nursery crops.

The rate used in the 1999 pansy and poinsettia trials cannot be determined and was reported as "per label". As a result, it was difficult to extrapolate the results to the proposed rate. However, the geranium trial does show that RootShield provided some protection from root rot caused by *Pythium ultimum*. A drench application of RootShield at 31 g/100 L resulted in significantly higher dry root weight compared to controls. Both plant height and fresh shoot weights of RootShield-treated plants were comparable to the uninoculated healthy controls.

The suppression of root diseases caused by *Pythium* spp., *Rhizoctonia* spp. and *Fusarium* spp. on all greenhouse ornamentals is already included in the current label. While the performance of RootShield on outdoor ornamentals may vary from its performance under greenhouse conditions, it is reasonable to assume that disease will be suppressed when RootShield is used in potted shrubs grown outdoors. The claim of suppression of root diseases on ornamentals grown outdoors is supported based on the currently registered use pattern.

5.1.1.2 Acceptable Efficacy Claims for RootShield Granules Biological Fungicide

No data or rationale were provided to support the claim of suppression of root diseases caused by *Pythium* spp., *Rhizoctonia* spp. and *Fusarium* spp. on greenhouse pepper and outdoor ornamental crops. However, root diseases that affect greenhouse cucumbers and tomatoes would also affect greenhouse peppers in a similar manner. Therefore, the claim on greenhouse peppers is supported by extrapolation from the current use pattern that includes greenhouse tomatoes and cucumbers.

The proposed use on outdoor nursery crops is supported because the rate and application method are the same as those of the currently registered use pattern of RootShield Granules Biological Fungicide. Considering the mode of action of RootShield, it is expected that the same level of control (suppression) will result for outdoor uses provided the directions on the current label are followed.

The claim for suppression of root diseases caused by *Pythium* spp., *Rhizoctonia* spp. and *Fusarium* spp. in greenhouse pepper is supported. The claim for suppression of root diseases caused by *Pythium* spp., *Rhizoctonia* spp. and *Fusarium* spp. in outdoor nursery crops is supported.

5.2 Phytotoxicity to Target Plants

No phytotoxicity was reported in host plants in any of the trials. It should be noted, however, that not all varieties of ornamental plants can be tested in efficacy trials. Therefore, it is recommended that RootShield be tested on a small sample of plants before applying the product on a commercial scale.

5.3 Economics

No market analysis was done for this submission.

5.4 Sustainability

5.4.1 Survey of Alternatives

The alternative ingredients registered for suppression of diseases caused by *B. cinerea*, *Pythium* spp., *Rhizoctonia* spp. and *Fusarium* spp. are presented in Appendix I, Table 2. There are no alternatives available for root diseases caused by *Pythium* spp., *Rhizoctonia* spp. and *Fusarium* spp. on greenhouse peppers.

5.4.2 Compatibility with Current Management Practices Including Integrated Pest Management

Please refer to Section 7.3 of Proposed Regulatory Decision Document PRDD2007-01, RootShield Biological Fungicide, *Trichoderma harzianum* Rifai strain KRL-AG2.

Fourteen fungicides were tested in an in vitro (agar plate) study on the compatibility of RootShield with conventional fungicides. The results show that RootShield was fully compatible with nine, moderately compatible with one and not compatible with four fungicide active ingredients.

5.4.3 Information on the Occurrence or Possible Occurrence of the Development of Resistance

Please refer to Section 7.5 of PRDD2007-01.

5.4.4 Contribution to Risk Reduction and Sustainability

Please refer to section 7.4 of PRDD2007-01.

6.0 Pest Control Product Policy Considerations

6.1 Toxic Substances Management Policy Considerations

The management of toxic substances is guided by the federal government's *Toxic Substances Management Policy*, which puts forward a preventive and precautionary approach to deal with substances that enter the environment and could harm the environment or human health. The policy provides decision makers with direction and sets out a science-based management framework to ensure that federal programs are consistent with its objectives. One of the key management objectives is virtual elimination from the environment of toxic substances that result predominantly from human activity and that are persistent and bioaccumulative. These substances are referred to in the policy as Track 1 substances.

In its review, the PMRA took into account the federal Toxic Substances Management Policy and followed its Regulatory Directive DIR99-03, *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy*. Substances associated with its use were also considered, including microcontaminants in the technical product, RootShield Technical Grade Active Ingredient, and formulators in the manufacturing-use products RootShield HC-Biological Fungicide Wettable Powder and RootShield Granules Biological Fungicide. The PMRA has reached the following conclusions:

- RootShield Biological Fungicide Technical Grade Active Ingredient does not meet the Track 1 criteria because the active ingredient is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products. No formulators, contaminants or impurities are present in the end-use products that would meet the Toxic Substances Management Policy Track 1 criteria. Therefore, the outdoor use of RootShield HC-Biological Fungicide Wettable Powder and RootShield Biological Fungicide Granules is not expected to result in Track 1 substances entering the environment.

6.2 Formulators and Contaminants of Health or Environmental Concern

RootShield Biological Fungicide Technical Grade Active Ingredient does not contain any contaminants of health or environmental concern identified in *Canada Gazette Part II, Volume 139, Number 24, pages 2641-2643: List of Pest Control Product Formulators and Contaminants of Health or Environmental Concern*.

The end-use products, RootShield HC-Biological Fungicide Wettable Powder and RootShield Granules Biological Fungicide, do not contain any contaminants of health or environmental concern identified in *Canada Gazette Part II, Volume 139, Number 24, pages 2641-2643: List of Pest Control Product Formulators and Contaminants of Health or Environmental Concern*.

7.0 Summary

7.1 Methods for Analysis of the Micro-organism as Manufactured

The product characterization data for the RootShield Biological Fungicide technical grade active ingredient and end-use products, RootShield HC-Biological Fungicide Wettable Powder and RootShield Granules, were judged to be adequate to assess their potential human health and environmental risks. The technical material was fully characterized and the specifications were supported by the analyses of a sufficient number of batches. Previously submitted storage stability data were sufficient to support a shelf life of 12 months at 2–5°C.

7.2 Human Health and Safety

The acute toxicity and infectivity studies previously submitted in support of *Trichoderma harzianum* strain KRL-AG2 were determined to be sufficiently complete to permit a decision on registration of outdoor uses. *Trichoderma harzianum* strain KRL-AG2 was of low toxicity in the rat when administered via oral and pulmonary exposure routes and was not pathogenic or infective via the pulmonary and intravenous injection exposure routes. In the pulmonary and intravenous injection infectivity studies, a pattern of clearance was established by day 21.

A rationale previously submitted to waive the requirement for a primary dermal irritation study for RootShield HC-Biological Fungicide Wettable Powder and RootShield Granules was acceptable.

The eye irritation potential of *Trichoderma harzianum* strain KRL-AG2 was tested by observing the effects of a single dose of approximately 10^8 CFU of the test organism to one eye in each of six New Zealand White rabbits. Based on the results of this study, *Trichoderma harzianum* strain KRL-AG2 at a dose of approximately 10^8 CFU per rabbit eye was determined to be minimally irritating and did not require signal words on the product labels. Because the test substance was a pure powder preparation and the end-use products contain formulants that are eye irritants, signal words on the product labels are required.

When handled according to the label instructions, the potential for dermal, eye and inhalation exposure for applicators, mixer/loaders and handlers exists, with the primary source of exposure to workers being dermal. Precautionary statements on product labels and personal protective equipment will adequately mitigate the risks from exposure.

While *Trichoderma harzianum* strain KRL-AG2 has the potential to be a sensitizing agent, inhalation and dermal exposure are not concerns if the required dust/mist filtering respirator and appropriate personal protective equipment stipulated on the end-use product label is worn by applicators, mixer/loaders, and handlers. Furthermore, precautionary labelling will alert users of the potential sensitization hazard of the end-use products.

Given that *Trichoderma harzianum* is an indigenous soil microorganism, it is unlikely the outdoor use of the wettable powder or granular formulation of RootShield Biological Fungicide will result in an increase in residues on treated food/feed stuffs considerably higher than natural conditions. In addition, *Trichoderma harzianum* strain KRL-AG2 demonstrated no oral toxicity and was not pathogenic or infective via pulmonary or intravenous exposure routes at the maximum dose tested in the Tier I acute toxicity/infectivity studies. *Trichoderma harzianum* strain KRL-AG2 is not known to produce any secondary metabolites beyond the level of those produced by naturally occurring strains and there have been no reports of adverse effects to humans from natural populations of *T. harzianum*. Therefore, negligible to no risk is expected for the general population, including infants and children, or animals from residues in or on agricultural commodities. Consequently, the establishment of an MRL is not required for

Trichoderma harzianum strain KRL-AG2 as defined under Division 15, Section B.15.002 of the Food and Drugs Regulations.

7.3 Environmental Risk

Previously submitted waiver rationales were used to address the environmental toxicology requirements. These rationales were based on the ubiquitous nature of *T. harzianum*, the lack of reported adverse effects in the literature, the inability of *Trichoderma harzianum* to become established in unpolluted aquatic environments, and the limited persistence expected of secondary metabolites that may be produced.

Sufficient information and data regarding environmental fate and ecotoxicological effects were previously submitted to support registration of new uses for RootShield Technical containing *Trichoderma harzianum* KRL-AG2 and two end-use products, RootShield Granules Biological Fungicide and RootShield HC-Biological Fungicide Wettable Powder. These products are intended for the suppression of root and foliar diseases in greenhouse and agricultural crops, and nursery stock. No additional studies or data are required to complete the environmental risk assessment.

As a precaution, standard label statements will prohibit handlers from contaminating aquatic habitats and provide instructions on preventing runoff from treated fields containing this product from entering lakes, streams, ponds or other water bodies.

7.4 Value

RootShield Granules Biological Fungicide and RootShield HC-Biological Fungicide Wettable Powder suppress several plant diseases and contribute to management of plant diseases that may otherwise require frequent application of fungicides for disease control. The use of these RootShield products will help reduce fungicide use in greenhouses and the field, with consequent reduction in occupational, dietary, and environmental exposure to chemical fungicides.

8.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, is proposing amended full registration for the sale and use of RootShield Technical Biological Fungicide and RootShield HC-Biological Fungicide Wettable Powder and RootShield Granules Biological Fungicide containing the technical grade active ingredient *Trichoderma harzianum* Rifai strain KRL-AG2, to control a variety of fungal diseases on greenhouse peppers, tomato and strawberry (in both greenhouses and the field), field lettuce and outdoor nursery plants as well as bean, pea, lima bean, lentil and soybean seeds.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

List of Abbreviations

°C	degree(s) Celcius
a.i.	active ingredient
bw	body weight
CCNS	cycloheximide, chlortetracycline, nystatin, streptomycin sulfate and Igepal
CFU	colony forming unit
cm	centimetre
g	gram
kg	kilograms
L	litre
LD ₅₀	lethal dose 50%
mg	milligram
mL	millilitre
MPCA	microbial pest control agent
MRL	maximum residue limit
NIOSH	National Institute of occupational Safety and Health
PMRA	Pest Management Regulatory Agency
PPE	personal protective equipment
ppm	part per million

Appendix I Tables and Figures

For tables on toxicity and infectivity of *Trichoderma harzianum* strain KRL-AG2 and associated end-use products, RootShield HC-Biological Fungicide Wettable Powder And RootShield Granules Biological Fungicide, as well as tables on toxicity to non-target terrestrial organisms refer to PRDD 2007-01, *RootShield Biological Fungicide, Trichoderma harzianum Rifai strain KRL-AG2*.

Table 1 Toxicity to Non-Target Aquatic Organisms

Organism	Exposure	Test Substance	Endpoint Value / Comments
Fish	Acute	Waiver rationale submitted in lieu of data	The waiver request was supported with a brief literature review. The review described the natural occurrences of species of <i>Trichoderma</i> . Although ubiquitous in most terrestrial environments, few references cited instances of recovery of any species of <i>Trichoderma</i> from aqueous environments. All but one of those occurrences involved polluted waters. One reference reported the isolation of <i>Trichoderma harzianum</i> from a marine sponge. Based on <i>T. harzianum</i> 's apparent inability to establish itself in unpolluted waters and on the absence of adverse effects to fish in the published literature, this request for a waiver was ACCEPTED .
Arthropods	Acute	Waiver rationale submitted in lieu of data	The waiver request was supported with published literature. As noted above, <i>Trichoderma</i> species are rarely isolated from aqueous environments. Few references have reported their occurrence and only one of those occurrences involved unpolluted waters. This reference reported the isolation of <i>Trichoderma harzianum</i> from a marine sponge. Based on <i>T. harzianum</i> 's apparent inability to establish itself in unpolluted waters and on the absence of adverse effects to arthropods in the published literature, this request for a waiver was ACCEPTED .
Non-Arthropods	Acute	Waiver rationale	<p>The applicant previously submitted an acceptable waiver rationale based on the absence of literature reporting adverse effects on non-arthropod invertebrates.</p> <p>A literature search found one reference in which eastern oyster (<i>Crastrea gigas</i>) embryos exposed to sediment extracts displayed developmental effects (a protruding mantle) by the D-larval stage. Analysis of the extracts implicated peptaibols (metabolites produced by many strains of <i>T. harzianum</i>, including strain KRL-AG2) in the disruption of embryogenesis but were not thought to be solely responsible for the effects seen. In the same article, <i>Trichoderma longibrachiatum</i> was isolated from the blue oyster and</p>

Appendix I Tables and Figures

For tables on toxicity and infectivity of *Trichoderma harzianum* strain KRL-AG2 and associated end-use products, RootShield HC-Biological Fungicide Wettable Powder And RootShield Granules Biological Fungicide, as well as tables on toxicity to non-target terrestrial organisms refer to PRDD 2007-01, *RootShield Biological Fungicide, Trichoderma harzianum Rifai strain KRL-AG2*.

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Arthropods	Acute	Waiver rationale submitted in lieu of data	The waiver request was supported with published literature. As noted above, <i>Trichoderma</i> species are rarely isolated from aqueous environments. Few references have reported their occurrence and only one of those occurrences involved unpolluted waters. This reference reported the isolation of <i>Trichoderma harzianum</i> from a marine sponge. Based on <i>T. harzianum</i> 's apparent inability to establish itself in unpolluted waters and on the absence of adverse effects to arthropods in the published literature, this request for a waiver was ACCEPTED .
Non-Arthropods	Acute	Waiver rationale	<p>The applicant previously submitted an acceptable waiver rationale based on the absence of literature reporting adverse effects on non-arthropod invertebrates.</p> <p>A literature search found one reference in which eastern oyster (<i>Crassostrea gigas</i>) embryos exposed to sediment extracts displayed developmental effects (a protruding mantle) by the D-larval stage. Analysis of the extracts implicated peptaibols (metabolites produced by many strains of <i>T. harzianum</i>, including strain KRL-AG2) in the disruption of embryogenesis but were not thought to be solely responsible for the effects seen. In the same article, <i>Trichoderma longibrachiatum</i> was isolated from the blue oyster and</p>

Organism	Exposure	Test Substance	Endpoint Value / Comments
			cultured. Peptaibols, extracted and identified from cultures of the fungus, elicited embryotoxic effects in assays performed on oyster embryos. Although <i>Trichoderma harzianum</i> strain KRL-AG2 is known to produce similar peptaibols as those investigated in the above study, <i>Trichoderma harzianum</i> is not expected to establish itself in aquatic environments in the event of run off or spray drift from treated fields.
Aquatic Plants	Acute	Waiver rationale submitted in lieu of data	The waiver request was supported with literature searches. As previously noted, <i>Trichoderma</i> species are rarely reported to occur on living plants and in unpolluted aqueous environments. Given that no adverse effects to aquatic plants were reported in the published literature, this request for a waiver was ACCEPTED .

Table 2 Alternative Active Ingredients Registered for Control or Suppression of Claimed Diseases on the RootShield HC-Biological Fungicide Wettable Powder Accepted Label

Crop	Disease Claim	Active Ingredient
Strawberry	Grey mould	Captan, chlorothalonil, boscalid, vinclozolin
Lettuce	Botrytis blight	Iprodione, ferbam, fenhexamid, boscalid, <i>Bacillus subtilis</i> strain QST713, dicloran, <i>Gliocladium catenulatum</i> strain J1446, zineb
Ornamentals	Botrytis blight	Fenhexamid, copper, <i>Bacillus subtilis</i> strain QST713, <i>Gliocladium catenulatum</i> strain J1446
Tomato	Botrytis stem canker	Fenhexamid, boscalid, <i>Bacillus subtilis</i> strain QST713, <i>Gliocladium catenulatum</i> strain J1446
Beans	Seed rot caused by <i>Pythium</i> spp., <i>Fusarium</i> spp. and <i>Rhizoctonia</i> spp.	Carbathiin, thiram, trifloxystrobin, azoxystrobin
Pea	Seed rot caused by <i>Pythium</i> spp., <i>Fusarium</i> spp. and <i>Rhizoctonia</i> spp.	Carbathiin, thiram, metalaxyl
Lima bean	Seed rot caused by <i>Pythium</i> spp., <i>Fusarium</i> spp. and <i>Rhizoctonia</i> spp.	Metalaxyl, fludioxonil, azoxystrobin, trifloxystrobin
Soybean	Seed rot caused by <i>Pythium</i> spp., <i>Fusarium</i> spp. and <i>Rhizoctonia</i> spp.	Carbathiin, thiram, fludioxonil, trifloxystrobin
Lentil	Seed rot caused by <i>Pythium</i> spp., <i>Fusarium</i> spp. and <i>Rhizoctonia</i> spp.	Carbathiin, thiram, trifloxystrobin
Greenhouse pepper	Root diseases caused by <i>Pythium</i> spp., <i>Fusarium</i> spp. and <i>Rhizoctonia</i> spp.	None
Ornamental crops	Root diseases caused by <i>Pythium</i> spp., <i>Fusarium</i> spp. and <i>Rhizoctonia</i> spp.	Trifloxystrobin (root rot caused by <i>Rhizoctonia solani</i>)

Table 3 Use (label) Claims Proposed by Applicant and Whether Acceptable or Unsupported

Label Claims Proposed by Registrant with Original Application	Accepted Label Claims	Unsupported Label Claims and Comments
Grey mould on strawberry (rate: 3.75–7.5 g/L)	Accepted with modification	Rate modified to 10 g/L since the proposed rates were not tested.
Botrytis stem canker on tomato (3.75–7.5 g/L)	Accepted with modification	Lower rate (3.75 g/L) supported. Higher rate (7.5 g/L) was not supported because it was not tested and no justification for the use of this rate was provided.
Botrytis blight on lettuce	Accepted with modification	Rate modified to 10 g/L since the proposed rates were not tested.
Botrytis blight on ornamentals (rate: 3.5–7.5 g/L)	Accepted with modifications	Supported for botrytis blight caused by <i>Botrytis cinerea</i> only. Only the higher rate (7.5 g/L) was supported since the lower rate (3.5 g/L) was not tested and use of 2.5 g/L provided inadequate disease suppression.
Root rot caused by <i>Pythium</i> spp., <i>Fusarium</i> spp. and <i>Rhizoctonia</i> spp. for all true seeds (seed treatment)	Accepted with modifications	Use as seed treatment supported at the proposed rate for the following legume species only: bean, pea, lentil, lima bean and soybean
Root diseases caused by <i>Pythium</i> spp., <i>Rhizoctonia</i> spp. and <i>Fusarium</i> spp. on greenhouse pepper	Accepted as proposed	
Root diseases caused by <i>Pythium</i> spp., <i>Rhizoctonia</i> spp. and <i>Fusarium</i> spp. on outdoor ornamental crops	Accepted as proposed	
Rhizoctonia diseases on outdoor ornamental crops	Not supported at this time	Insufficient data
<i>Botrytis</i> spp. on ornamental crops	Not supported at this time	Insufficient data
Dollar spot caused by <i>Sclerotinia homoeocarpa</i> on turf	Not supported at this time	Insufficient data

Organism	Exposure	Test Substance	Endpoint Value / Comments
			cultured. Peptaibols, extracted and identified from cultures of the fungus, elicited embryotoxic effects in assays performed on oyster embryos. Although <i>Trichoderma harzianum</i> strain KRL-AG2 is known to produce similar peptaibols as those investigated in the above study, <i>Trichoderma harzianum</i> is not expected to establish itself in aquatic environments in the event of run off or spray drift from treated fields.
Aquatic Plants	Acute	Waiver rationale submitted in lieu of data	The waiver request was supported with literature searches. As previously noted, <i>Trichoderma</i> species are rarely reported to occur on living plants and in unpolluted aqueous environments. Given that no adverse effects to aquatic plants were reported in the published literature, this request for a waiver was ACCEPTED .

Table 2 Alternative Active Ingredients Registered for Control or Suppression of Claimed Diseases on the RootShield HC-Biological Fungicide Wettable Powder Accepted Label

Crop	Disease Claim	Active Ingredient
Strawberry	Grey mould	Captan, chlorothalonil, boscalid, vinclozolin
Lettuce	Botrytis blight	Iprodione, ferbam, fenhexamid, boscalid, <i>Bacillus subtilis</i> strain QST713, dicloran, <i>Gliocladium catenulatum</i> strain J1446, zineb
Ornamentals	Botrytis blight	Fenhexamid, copper, <i>Bacillus subtilis</i> strain QST713, <i>Gliocladium catenulatum</i> strain J1446
Tomato	Botrytis stem canker	Fenhexamid, boscalid, <i>Bacillus subtilis</i> strain QST713, <i>Gliocladium catenulatum</i> strain J1446
Beans	Seed rot caused by <i>Pythium</i> spp., <i>Fusarium</i> spp. and <i>Rhizoctonia</i> spp.	Carbathiin, thiram, trifloxystrobin, azoxystrobin
Pea	Seed rot caused by <i>Pythium</i> spp., <i>Fusarium</i> spp. and <i>Rhizoctonia</i> spp.	Carbathiin, thiram, metalaxyl
Lima bean	Seed rot caused by <i>Pythium</i> spp., <i>Fusarium</i> spp. and <i>Rhizoctonia</i> spp.	Metalaxyl, fludioxonil, azoxystrobin, trifloxystrobin
Soybean	Seed rot caused by <i>Pythium</i> spp., <i>Fusarium</i> spp. and <i>Rhizoctonia</i> spp.	Carbathiin, thiram, fludioxonil, trifloxystrobin
Lentil	Seed rot caused by <i>Pythium</i> spp., <i>Fusarium</i> spp. and <i>Rhizoctonia</i> spp.	Carbathiin, thiram, trifloxystrobin
Greenhouse pepper	Root diseases caused by <i>Pythium</i> spp., <i>Fusarium</i> spp. and <i>Rhizoctonia</i> spp.	None
Ornamental crops	Root diseases caused by <i>Pythium</i> spp., <i>Fusarium</i> spp. and <i>Rhizoctonia</i> spp.	Trifloxystrobin (root rot caused by <i>Rhizoctonia solani</i>)

Table 3 Use (label) Claims Proposed by Applicant and Whether Acceptable or Unsupported

Label Claims Proposed by Registrant with Original Application	Accepted Label Claims	Unsupported Label Claims and Comments
Grey mould on strawberry (rate: 3.75–7.5 g/L)	Accepted with modification	Rate modified to 10 g/L since the proposed rates were not tested.
Botrytis stem canker on tomato (3.75–7.5 g/L)	Accepted with modification	Lower rate (3.75 g/L) supported. Higher rate (7.5 g/L) was not supported because it was not tested and no justification for the use of this rate was provided.
Botrytis blight on lettuce	Accepted with modification	Rate modified to 10 g/L since the proposed rates were not tested.
Botrytis blight on ornamentals (rate: 3.5–7.5 g/L)	Accepted with modifications	Supported for botrytis blight caused by <i>Botrytis cinerea</i> only. Only the higher rate (7.5 g/L) was supported since the lower rate (3.5 g/L) was not tested and use of 2.5 g/L provided inadequate disease suppression.
Root rot caused by <i>Pythium</i> spp., <i>Fusarium</i> spp. and <i>Rhizoctonia</i> spp. for all true seeds (seed treatment)	Accepted with modifications	Use as seed treatment supported at the proposed rate for the following legume species only: bean, pea, lentil, lima bean and soybean
Root diseases caused by <i>Pythium</i> spp., <i>Rhizoctonia</i> spp. and <i>Fusarium</i> spp. on greenhouse pepper	Accepted as proposed	
Root diseases caused by <i>Pythium</i> spp., <i>Rhizoctonia</i> spp. and <i>Fusarium</i> spp. on outdoor ornamental crops	Accepted as proposed	
<i>Rhizoctonia</i> diseases on outdoor ornamental crops	Not supported at this time	Insufficient data
<i>Botrytis</i> spp. on ornamental crops	Not supported at this time	Insufficient data
Dollar spot caused by <i>Sclerotinia homoeocarpa</i> on turf	Not supported at this time	Insufficient data

References

A. List of Studies/Information Submitted by Registrant

1.0 Chemistry

2.0 Human and Animal Health

3.0 Environment

4.0 Value

PMRA Document Number: 1308741

Reference: Part 10 - Value For A Plant Protection Product, Data Numbering Code: M10.0,M10.1,M10.2.2

PMRA Document Number: 1356327

Reference: Attachment 8-product Performance, Data Numbering Code: M10.2,M2.14

PMRA Document Number: 1356328

Reference: 1998, Evaluation Du Produit De Lutte Biologique Rootshield Contre Le Pythium Sur Culture De Tomate De Serre En Solutions Nutritives Et En Mousse De Tourbe. Data Numbering Code: M10.2.2

PMRA Document Number: 1356329

Reference: 1997, Disease Prevention In Greenhouse Tomato: An IPM Perspective. Attachment 9. Data Numbering Code: M10.2.2

PMRA Document Number: 1356335

Reference: Attachment 11-6. Integrated Flower Disease Management; 7. Major Greenhouse Flower Diseases, Data Numbering Code: M10.3.1,M2.14

PMRA Document Number: 1356336

Reference: Nature And Economics Of Disease Problem. Attachment 12, Data Numbering Code: M10.4.2

PMRA Document Number: 1356337

Reference: Current Crop Protection Tools. Attachment 13, Data Numbering Code: M10.4.3

B. Additional Information Considered

I) Published Information

1.0 Environment

PMRA Document Number: 1738535

Reference: Poirier, L., Quiniou, F., Ruiz N., Montagu, M., Amiard, J., and Pouchus Y. F. 2007. Toxicity Assessment of Peptaibols and Contaminated Sediments on *Crassostrea gigas* Embryos. Aquatic Toxicology 83:254B262. Data Numbering Code: M9.6

